Embryology and Seed Development in the Winged Bean, *Psophocarpus tetragonolobus*

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Abstract

In *Psophocarpus tetragonolobus* (L.) DC the anther is tetrasporangiate. Its wall development usually conforms to the Dicotyledonous type but occasionally to the Basic type. Simultaneous cytokinesis in microsporocytes results in tetrahedral microspore tetrads. The mature pollen grains are triporate and 2-celled with a reticulate exine. The mature ovule is campylotropous, bitegmic and crassinucellate. The micropyle is zig-zag and formed by both the integuments. The embryo sac development follows the monosporic Polygonum type. Fertilization is porogamous and triple fusion precedes syngamy. The endosperm development is of the Nuclear type and free-nuclear endosperm haustoria develop in both the micropylar, and chalazal parts. The first two divisions of the zygote are transverse producing a linear 4-celled proembryo, but the subsequent divisions are in various planes. At the early globular stage of the embryo, the suspensor cells become hypertrophied and haustorial. In mature seed, the inner integument remains 2 or 3 layered, but the outer becomes 15-30 layers and is particularly massive around the micropyle. The thick-walled palisade cells of the seed coat are derived from the outer epidermis of the outer integument, while all the other layers remain parenchymatous.

Introduction

Psophocarpus tetragonolobus (L.) DC., the winged bean, is a perennial climber of the tribe Phaseoleae of the legume subfamily Papilionoideae. The geographical origin of the species is uncertain with Africa (where all the other five species of the genus are indigenous), India, South East Asia (particularly Indonesia, where there are numerous local names for the plant), and Papua New Guinea having been suggested variously as possible places of origin (Hymowitz and Boyd, 1977). Hymowitz and Boyd (1977) themselves favoured a Papua New Guinea origin and stated that "it would be difficult to find another high rainfall-adapted tropical legume crop with as many desirable characteristics as *Psophocarpus tetragonolobus.*" Virtually all parts of the plant are edible. Besides the unripe fruits, which are popular as a green vegetable, the

leaves, flowers, seeds and tuberous roots are consumed as well. The protein and oil content (37% and 18% respectively) of the seeds compares favourably with that of soybeans, and even the leaves, flowers, pods and tubers have protein levels of 10-15% (Abbiw, 1990). The plants are also considered as a valuable source of green fodder and manure. Detailed embryological information, which might be useful in any attempts to improve this crop of considerable potential, has so far been lacking.

Materials and Methods

Flowering and fruiting materials of suitable ages were collected between 1990 and 1992 from glasshouse-grown plants at the Department of Botany, University of New England. These were fixed in FPA (formalin-propionic acid- 70% ethyl alcohol - 5:5:90 v/v) and stored after 48 hr in 70% ethyl alcohol. Following standard procedures of microtechnique (Sass, 1958), the specimens were embedded in Paraplast, sectioned, and stained in safranin and fast green. Permanent slides were made after dehydration in an ethyl alcohol series, treatment with Histoclear (a xylene substitute), and mounting in Eukitt.

Observations

Anther:

The anther is tetrasporangiate (Fig. 1A). Hypodermal archesporial cells in each sporangium divide periclinally to form the primary parietal and primary sporogenous cells (Fig 1B). The latter divide repeatedly in various directions and differentiate into microsporocytes. The primary parietal cells divide periclinally to form the outer and inner secondary parietal cells. Usually, only the outer secondary parietal cells continue further divisions whereas the inner do not (Fig. 1D). The derivatives of the outer secondary parietal cells constitute the endothecium and the two middle layers while the inner secondary parietal cells directly develop into the tapetum. Occasionally the inner secondary parietal cells also show mitosis (Fig. 1C) suggesting that although the anther wall formation usually conforms to the Dicotyledonous type (Davis, 1966), the Basic type may also occur. Rarely, the cells of a middle layer may further divide resulting in a third middle layer (Fig. 1E). The tapetum is secretory; its cells remain uninucleate and develop one or two large vacuoles before meiosis occurs in the microsporocytes (Fig.1F,G). The mature anther wall, lying under

the single-layered epidermis, consists of one layer each of endothecium and tapetum and 2 or 3 middle layers lying in between.

At the time of meiosis, the microsporocytes become ensheathed in callose. Simultaneous cytokinesis accompanies meiosis (Fig. 1H-J) and the resultant microspore tetrads are tetrahedral (Fig. 1G, K). Different stages of development, ranging from late prophase to the microspore tetrad phase, can be observed within the same flower bud. In addition, different microsporangia of the same anther may exhibit different stages of development though usually all microsporocytes within a single sporangium show synchrony. However, in one instance while the microsporocytes in the lower part of the sporangium were at the late prophase stage, those in the middle were at diakinesis, and in the upper part they were at metaphase I.

The microspore walls are formed by furrowing and the microspores are initially isolated from each other by callose (Fig. 1J,K). Dissolution of callose releases individual microspores into the sporangium by which time a thin exine and incipient pores become evident (Fig. 1L). At this stage, the tapetum is still intact though large vacuoles appear in the cells and the cytoplasm appears sparse. By then all but one of the middle layers disintegrate. The uninucleate microspore enlarges, the exine thickens, and its nucleus migrates to the periphery of a large central vacuole, giving the microspore the characteristic signet-ring appearance. Next the tapetum also degenerates while the cells of the endothecium enlarge and become radially elongated. The microspore undergoes mitosis to form the 2-celled pollen grain. In the anther wall fibrous thickenings appear in the endothecium and the single persistent middle layer still remains distinct (Fig. 1M). By the time the microspores become mature pollen grains, the tapetum and the remaining middle layer completely break down leaving only the fibrous endothecium and the epidermis, the cells of which accumulate brownish-staining crystals (Fig. 1N). The mature pollen grains are triporate, 2-celled, and show a reticulate exine.

Ovule:

Mature ovules are campylotropous, bitegmic and crassinucellate. Ovular primordia, 10-18 in number, are initiated as homogeneous hemispherical protuberances from the marginal placenta (Fig. 2A). All the ovules in an ovary develop synchronously. The primordia begin to undergo curvature even before the integuments arise and finally assume the campylotropous form (Fig. 2A-C).

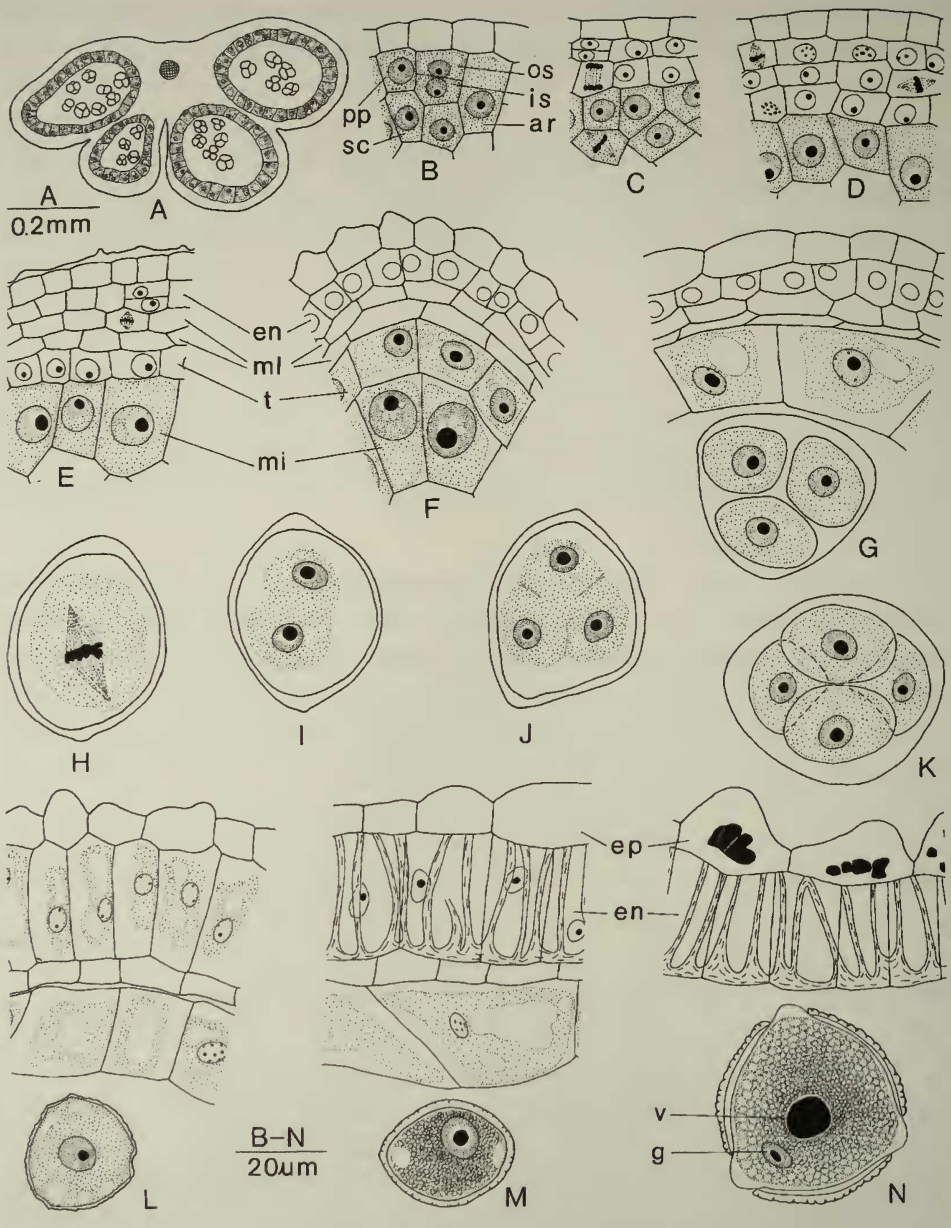

Fig. 1. ***Psophocarpus,*** **anther wall and pollen development.** All cross sections of anther. *A,* tetrasporangiate anther with pollen tetrads; *B-E,* stages in anther wall development; *F,* microspore mother cells and wall layers; *G,* anther wall at pollen tetrad; *H-K,* microsporogenesis; *L,M,* differentiation of the mature anther wall and pollen; *N,* anther wall and mature 2-celled pollen. (*ar,* archesporial cell; *en,* endothecium; *ep,* epidermis; *g,* generative nucleus; *is,* inner secondary parietal cell; *mi,* microsporocyte; ml, middle layer; *os,* outer secondary parietal cell; *sc,* sporogenous cell; *t,* tapetum; *v,* vegetative nucleus).

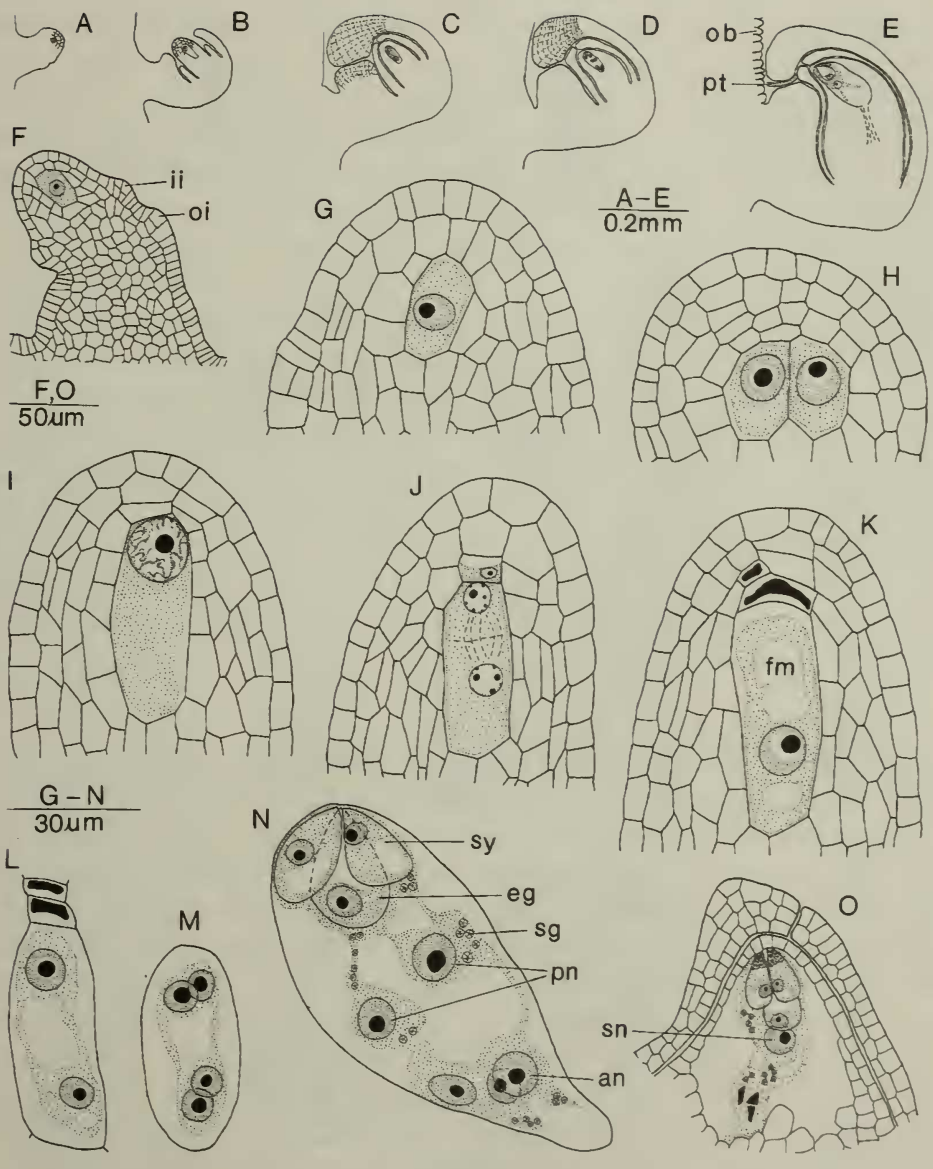

Fig. 2. ***Psophocarpus,*** **ovule and embryo sac development**. All longitudinal sections of ovule. *A-D,* stages in ovule development; *E,* pollen tube entering ovule; *F,* initiation of the inner and outer integuments; *G,* a single sporogenous cell; *H,* twin sporogenous cells; *I,* megasporocyte; J, megasporogenesis; *K-M,* megagametogenesis; *N,* mature female gametophyte; *O,* degeneration of antipodals in the female gametophyte. (*an,* antipodal; *eg,* egg; *fm,* functional megaspore; *ii,* inner integument; *ob,* obturator; *oi,* outer integument; *pn,* polar nuclei; *pt,* pollen tube; *sg,* starch grains; *sn,* secondary nucleus; *sy,* synergid).

Both integuments differentiate simultaneously from the nucellus at the sporogenous cell stage (Fig. 2F). However, the development of the inner integument is slower than that of the outer so that at the megasporocyte stage the outer integument of 3 layers encloses the inner integument of 2 or 3 layers and the nucellus (Fig. 2B). The outer integument continues to grow rapidly and completely envelops the nucellus at the end of megasporogenesis while the inner reaches only half its length. By the 2-nucleate stage of the female gametophyte, the inner integument, which is still 2 or 3 cells thick, reaches the micropyle while the cells of the outer integument undergo periclinal and anticlinal divisions in the micropylar part so that it becomes 8-10 cells thick at the tip (Fig. 2C). As the ovule matures, the tip of the outer integument continues to show repeated divisions to form a massive structure of 13-20 layers of cells, growing towards the funiculus and covering the inner integument and the micropylar end of the nucellus. As a consequence, the micropyle becomes zig-zag with the exostome surrounded by the outer integument and the endostome bounded on all sides by the inner integument of 2 or 3 layers of cells (Fig. 2D). Starch grains accumulate in the cells of the outer and inner integuments in the region surrounding the micropyle.

Usually one of the hypodermal cells differentiates into the archesporium which divides periclinally to form a primary parietal cell and a sporogenous cell (Fig. 2G). A single instance of twin sporogenous cells was observed (Fig. 2H). As the cytoplasm becomes denser and the nucleus enlarges, the sporogenous cell becomes the megasporocyte while the primary parietal cell divides giving rise to 2 or 3 nucellar layers above it (Fig. 2I). Cytokinesis following the first meiotic division produces a dyad, of which only the lower member undergoes meiosis II. Thus a linear triad is formed at the end of meiosis (Fig. 2J). The smaller upper dyad cell degenerates simultaneously with the middle megaspore, leaving only the chalazal megaspore to continue development (Fig. 2K). After three successive nuclear divisions a mature eight-nucleate female gametophyte is formed (Fig. 2L-N); its development thus conforming to the monosporic Polygonum type. As the female gametophyte matures, an egg flanked by two synergids - each with a filiform apparatus - differentiates at the micropylar end, the polar nuclei migrate toward the funicular side of the female gametophyte and the antipodals are organised in the chalazal region (Fig. 2N). Soon after formation, the two polar nuclei fuse into a large secondary nucleus which becomes located just below the egg (Fig. 2E,O).

During its development the female gametophyte rapidly increases in size at the expense of the nucellar tissue, especially at the sides and in the

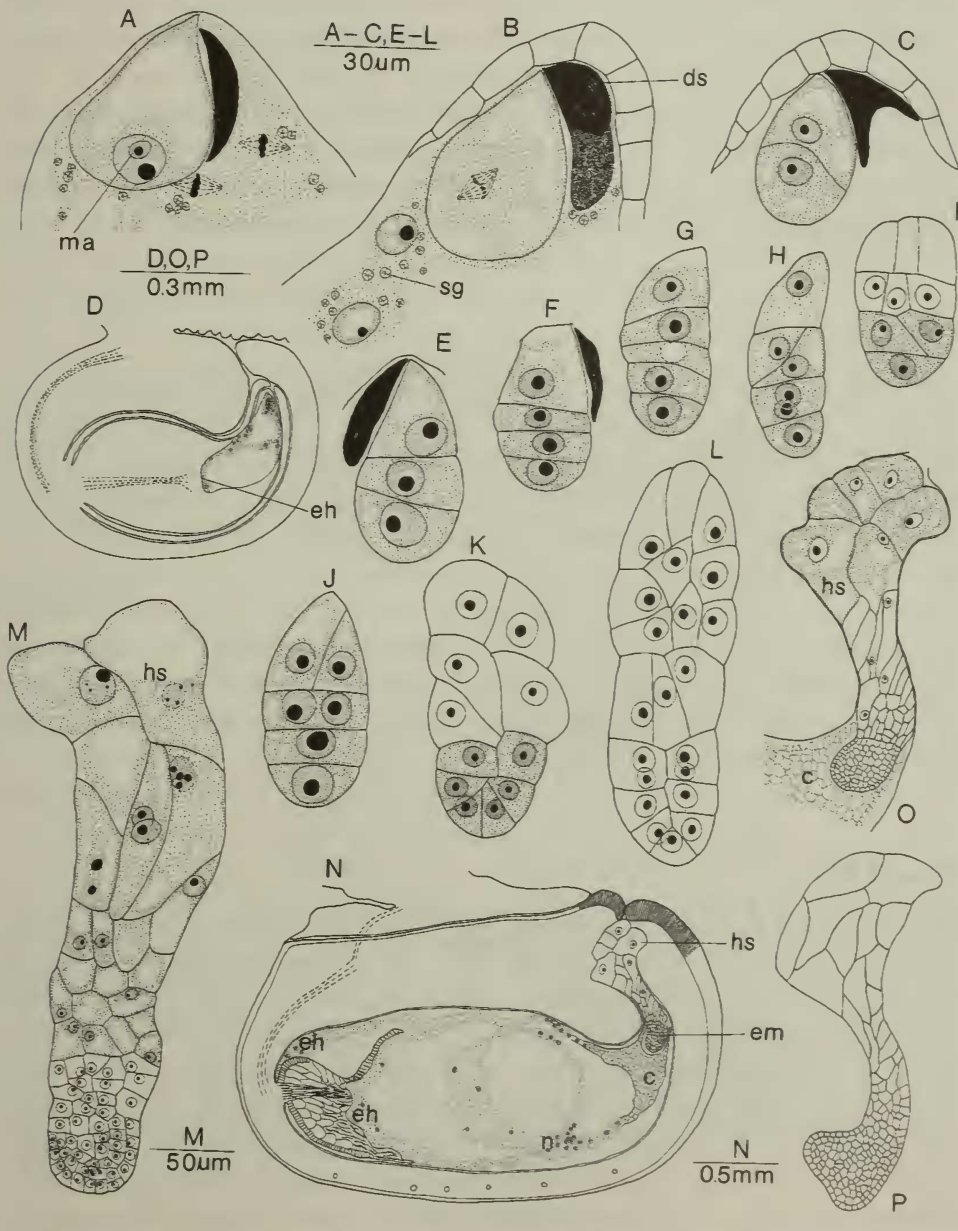

Fig. 3. ***Psophocarpus,*** **embryo development.** All longitudinal sections of seed. *A,* division of endosperm nucleus before syngamy; *B,C,* division of zygote; *D,* linear 4-celled proembryo and nuclear endosperm showing beginning of endosperm haustoria; *E-M,* stages in the development of globular embryo, note the extensive haustorial suspensor; *N,* section of whole seed showing globular embryo and endosperm haustoria; *O,* globular embryo; *P,* early heart-shaped embryo. (*c,* cellular endosperm; *ds,* degenerating synergid; *eh,* endosperm haustorium; *em,* embryo; *hs,* haustorial suspensor; *ma,* male gamete; *n,* endosperm nuclei; *sg,* starch grains).

micropylar region. Hence the mature female gametophyte is bordered by the inner integument on the sides, the single-layered nucellar epidermis at the micropylar end, and the hypostase in the chalazal region. An endothelium is not differentiated. Starch grains are first deposited in the nucellar epidermis at the 4-nucleate female gametophyte stage. They become abundant in not only the two integuments but also the mature female gametophyte (Fig. 2N,O) and persist even after fertilisation.

Fertilisation:

Pollen tubes grow along the placenta towards the nucellus through the micropyle (Fig. 2E, 4A). The epidermal cells of the placenta become papillate, assume a glandular appearance, and possibly act as a a pollen tube guide. The entry of the pollen tube is porogamous and through one of the synergids; the other synergid degenerates soon after fertilization. One male gamete fuses with the secondary nucleus to form the primary endosperm nucleus whereas the second enters the egg but does not immediately fuse with its nucleus. The two nuclei within the egg remain separate while the primary endosperm nucleus divides to form free endosperm nuclei (Fig. 3A). Syngamy occurs after the formation of eight endosperm nuclei.

Endosperm:

The development of the endosperm is of the Nuclear type. Just before the primary endosperm nucleus divides, the embryo sac enlarges significantly and large starch grains accumulate, particularly in the micropylar region. At least the first three mitotic divisions are synchronous. The free nuclei, resulting from repeated divisions, mainly occupy the micropylar region and along the periphery of the enlarging endosperm in the vicinity of the funiculus (Fig. 3D). At the 16 nucleate stage of the endosperm, the zygote divides (Fig. 3B). At the time of a linear proembryo there are about 60 endosperm nuclei. As the proembryo develops, the number of free nuclei continues to increase rapidly.

A tubular coenocytic haustorium develops from the upper end of the endosperm on the side of the funiculus. It grows towards the chalazal nucellar tissue causing many of the cells to degenerate (Fig. 3D, 4B). The narrow haustorium, with a sac-like tip, is discernible at the linear proembryo stage and becomes very distinct by the early globular stage. As the embryo develops, the haustorium grows in between the inner integument and the nucellus (Fig. 3N). In addition, the chalazalmost free-nuclear part of the

endosperm also shows haustorial activity. Both haustoria remain free-nuclear throughout the life of the endosperm.

The main body of the endosperm remains completely free-nuclear until the early globular embryo stage. The number of nuclei counted at this stage was 658 with 274 situated mainly in the micropylar quarter of the endosperm and showing either mitotic metaphase or anaphase. The remaining occur as free nuclei mostly along the periphery of the endosperm. Cell-formation in the endosperm begins from the micropylar region and proceeds towards the chalazal part at the late globular embryo stage (Fig. 4C). By now, because of the breakdown of the nucellus, the endosperm is completely surrounded by the inner integument except at the chalazal region where a butt of the nucellus persists until the cotyledons differentiate in the embryo. The endosperm is almost totally consumed by the embryo in the mature seed and the reserve foods are transferred to the cotyledons.

Embryo:

The zygote enlarges and divides after the formation of 16 nuclei in the endosperm (Fig. 3B). The first division is transverse forming an apical cell and a basal cell after which both further divide transversely to form a linear 4-celled proembryo (Fig. 3C, E, F). The next division may be transverse in one of the cells forming a linear 5-celled proembryo (Fig. 3G); or vertical and oblique in one or more cells (Fig. 3H-K). Subsequent oblique divisions result in the early globular embryo while the derivatives of the basal cell continue to divide in an irregular and variable manner to produce the cells of the suspensor (Fig.3K, L). The suspensor cells become hypertrophied and elongate significantly, extending from the embryo sac into the erstwhile endostome and finally touching the outer integument (Fig. 3M-O). By the heart-shaped stage of the embryo (Fig. 3P), the endosperm is mostly used up. The massive suspensor eats into the integuments and apparently assumes a major, if not the sole, responsibility for the continued nutrition of the embryo.

Seed:

The fertilised female gametophyte undergoes gross enlargement at the expense of cells in the micropylar part of the nucellus so that only 7 or 8 nucellar epidermal cells eventually persist. The cells of the inner integument divide anticlinally and undergo radial elongation. Meanwhile, the endosperm haustoria rapidly disorganise and destroy the chalazal nucellus which is gradually resorbed, thus increasing the size of the endosperm. At the linear 4-celled stage of the proembryo, the endosperm is bordered by the inner integument along half its length (Fig. 3D); while by the late globular embryo stage the entire length

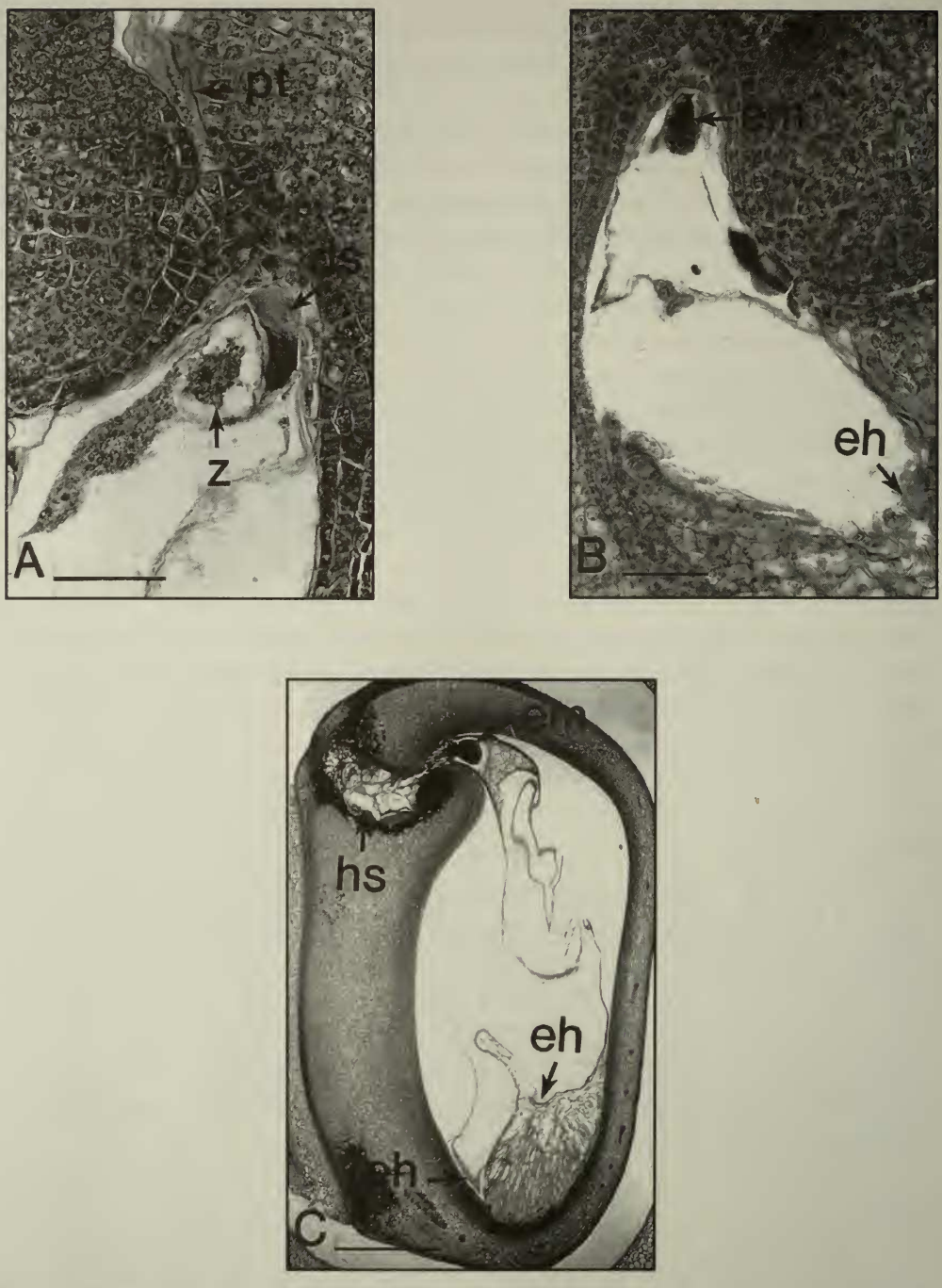

Fig. 4. ***Psophocarpus,*** **longitudinal sections of seeds**. *A,* division of zygote with degenerating synergid and pollen tube at the micropyle; *B,* linear 4-celled proembryo with nuclear endosperm forming haustorium; *C,* globular embryo with haustorial suspensor and endosperm haustoria. (*ds,* degenerating synergid; *eh,* endosperm haustorium; *em*, embryo; *hs,* haustorial suspensor; *pt,* pollen tube; *z,* zygote. Scale: *A,B* = 40mm; *C* = 0.5mm).

except the chalaza is lined by the inner integument (Fig. 3N). Throughout this development the inner integument continues to divide anticlinally to expand its length but remains 2 or 3 cells thick. The outer integument, on the other hand, becomes 15-30 layers thick through both anticlinal and periclinal divisions and is particularly massive around the micropylar region (Fig. 4C). Further, the outer epidermal cells of the outer integument divide anticlinally, the resultant cells elongate, lose their nuclei and differentiate into a palisade tissue of thick-walled columnar cells. The cells below the epidermis remain parenchymatous (Fig. 4C) without showing the differentiation of a layer of hour-glass cells as in certain other legumes.

Discussion

Dicotyledonous type of anther wall development was the only type recorded in the family (Davis, 1966; Prakash, 1987) until the present report of the occasional presence of the Basic type as well. Besides the secretory tapetum of uninucleate cells, the anther wall occasionally shows three middle layers which is one layer more than that reported in the other members of the family (Prakash, 1987). The morphology of the ovule and its development reaffirm the earlier observations on the family (Prakash, 1987; Prakash and Chan, 1976; Prakash and Herr, 1979). The female gametophyte development conforms to the monosporic Polygonum type, which seems to be a common feature throughout the Leguminosae - except for members of the Australian endemic papilionoid tribe Mirbelieae which exhibit a variety of novel patterns (Cameron and Prakash, 1993). Also, the antipodals are normal in *P. tetragonolobus* unlike certain Australian genera of the tribes Indigofereae, Bossiaeeae and Mirbelieae which possess giant antipodals (Cameron and Prakash, 1990). An instance of twin sporogenous cells has been observed; and this too is known in the family (Rembert, 1969b; Prakash, 1987). Presumably only one sporogenous cell continues development because twin female gametophytes, which have been recorded in certain other members of the family, have never been found in the present material. Often, during megasporogenesis, the second division of the micropylar dyad is arrested and a triad is formed as in *Cassia* (Rembert, 1969a), and *Vicia villosa* (Rembert, 1969b). An integumentary endothelium, which occurs in many other members of the family (Prakash, 1987), does not seem to differentiate in any member of the tribe Phaseoleae. However, an endothelium of nucellar origin has been reported in *Phaseolus aconitifolius* (Deshpande and Bhasin, 1974).

The egg nucleus fuses with the male gamete only after the division of the primary endosperm nucleus. This delayed syngamy has been described in

one other legume i.e., *Acacia leucophloea* (Ugemuge,1982), in which it is achieved after the formation of four endosperm nuclei. As in all other legumes studied so far, the endosperm is of the Nuclear type and it becomes cellular only after several hundred free nuclei are formed. The presence of a chalazal haustorium which remains free-nuclear even after cell-formation had occurred in the micropylar half of the endosperm is known in several legumes (Johri and Garg, 1959) including *Atylosia*, *Glycine*, *Teramnus* (Rau, 1953) *Canavalia*, *Hardenbergia violacea*, *Kennedia rubicunda* and *Vandasia retusa* (Cameron, 1988) of the tribe Phaseoleae. However, in *Psophocarpus* there is an additional tubular lateral haustorium which also remains coenocytic. The zygote divides when there are 16 free endosperm nuclei as has also been reported in *Acacia leucophloea* (Ugemuge,1982).

As in many other groups of the Leguminosae (Lersten, 1983), in Phaseoleae also the morphology of the suspensor is variable; from being filamentous in species of *Erythrina* (McNaughton, 1976) to massive in *Phaseolus*. Cameron (1988) found a short suspensor in *Glycine clandestina, Hardenbergia violacea* and *Kennedia rubicunda* but a long, filamentous, uniseriate suspensor of inflated cells in *Canavalia*. However, unlike *P. tetragonolobus*, in most species the suspensor is contained wholly within the endosperm.

The pattern of embryogeny in *Psophocarpus tetragonolobus* is essentially similar to that in *Glycine* (Ho, 1963; Souèges, 1949). The structure of the seed coat is similar to that described for the family by Corner (1951, 1976) who believed that the functioning of the exposed surface as a protective layer suggests a primitive construction. However, unlike certain other members of the family (Corner, 1976; Prakash and Chan, 1976), a layer of hour glass cells is not distinguished in the mesophyll of the winged bean seed.

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